REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
01-09-2011	Conference Proceeding	-

4. TITLE AND SUBTITLE

RETHINKING CHLOROPHYLL RESPONSES TO STRESS: FLUORESCENCE AND REFLECTANCE REMOTE SENSING IN A COASTAL ENVIRONMENT

5a. CONTRACT NUMBER
W911NF-10-1-0433

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
622784
611102

6. AUTHORS

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
57264-EV.4

12. DISTRIBUTION AVAILIBILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

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14. ABSTRACT

Chlorophyll fluorescence and hyperspectral reflectance were used to evaluate physiological responses to two common stressors in coastal environments. Chlorophyll content is one indicator of drought and salinity vegetation stress because of its direct role in the photosynthetic process and electron transport. Recent advances in fluorescence spectroscopy have led to the development of numerous reflectance indices that estimate fluorescence emission of vegetation for mapping vegetation stress as chlorophyll content and/or carotenoid content changes.

15. SUBJECT TERMS

Chlorophyll fluorescence, canopy reflectance, remote sensing, hyperspectral imagery, plant stress

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Donald Young
UU	UU	UU	UU		19b. TELEPHONE NUMBER: 804-828-1562

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Our results indicate that some species do not breakdown chlorophyll during periods of stress, which may be an adaptive in a highly dynamic environment. Variations in PRI were not related to changes in chlorophyll content or the carotenoids/chlorophyll ratio. PRI is an indicator of chronic salinity stress and may be used as an early signal for associated changes due to sea-level rise associated with climate change. Remote sensing of vegetation allows for providing biophysical measurements across landscapes with limited access. However, not all species respond via chlorophyll changes with moderate to high levels of stress and this must be considered as remote sensing of plant signals is applied globally across species.

Conference Name: 4th INTERNATIONAL WORKSHOP ON REMOTE SENSING OF VEGETATION FLUORESCENCE, VAL

Conference Date: November 15, 2010

RETHINKING CHLOROPHYLL RESPONSES TO STRESS: FLUORESCENCE AND REFLECTANCE REMOTE SENSING IN A COASTAL ENVIRONMENT

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ABSTRACT

Chlorophyll fluorescence and hyperspectral reflectance were used to evaluate physiological responses to two common stressors in coastal environments. Chlorophyll content is one indicator of drought and salinity vegetation stress because of its direct role in the photosynthetic process and electron transport. Recent advances in fluorescence spectroscopy have led to the development of numerous reflectance indices that estimate fluorescence emission of vegetation for mapping vegetation stress as chlorophyll content and/or carotenoid content changes. Photoprotection via xanthophyll changes and non-photochemical quenching (qN) have been well correlated to the photochemical reflectance index (PRI) which has been successfully applied at multiple scales and most commonly associated with drought stress. Our objective was to evaluate chlorophyll responses to drought and salinity induced stress in a common shrub species in laboratory experiments and relate to field collected data.

Our results indicate that some species do not breakdown chlorophyll during periods of stress, which may be an adaptive in a highly dynamic environment. Variations in PRI were not related to changes in chlorophyll content or the carotenoids/chlorophyll ratio. PRI is an indicator of chronic salinity stress and may be used as an early signal for associated changes due to sea-level rise associated with climate change. Remote sensing of vegetation allows for providing biophysical measurements across landscapes with limited access. However, not all species respond via chlorophyll changes with moderate to high levels of stress and this must be considered as remote sensing of plant signals is applied globally across species.

1. INTRODUCTION

The physiological condition of plants is indicative of plant productivity, adaptability to stress, and a general indication of the environment in which they grow [1]. Early detection through remote sensing could identify plant stress at larger spatial and temporal scales, before visible effects are apparent [1]. The success of remote sensing is predicated on the accurate identification and degree of stress. Stress may be apparent in morphological and physiological characteristics, which represent integrated responses to multiple environmental factors.

Estimation of pigments via remote sensing methods is of interest because it is a rapid, nondestructive way of assessing vegetative health, productivity, etc. Improvements of indices have been made that can analyze stress induced changes in photosynthetic pigments, especially chlorophylls and carotenoids. The chlorophylls (Chl *a* and Chl *b*) are essential pigments for the conversion of light energy to stored chemical energy. The amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content; thus, chlorophyll content can directly determine photosynthetic potential and primary production [2]. Plants have different mechanisms to dissipate excess light safely under environmental stress to avoid photoinhibition and photooxidation [3]. Chlorophyll fluorescence is the production of red and far red light in photosynthetic tissues upon excitation with light in the visible spectrum [1]. The percentage of absorbed light used in photosynthesis or dissipated as heat can be estimated by chlorophyll fluorescence parameters, and is directly related to plant physiological processes [3].

Chlorophyll content has been an important indicator of vegetation stress because of its direct role in the photosynthetic process and electron transport, and several reflectance indices have been demonstrated to accurately estimate total chlorophyll content and for use at different scales [1-2]. Declines in photosynthesis that occur with stress are generally accompanied by reductions in leaf chlorophyll concentrations as a protective mechanism to adjust photosynthetic machinery [4]. More recently focus has shifted to include carotenoid pigments because of the relationship with energy dissipation through the xanthophyll cycle, which is an important photoprotective mechanism and linked to photosynthetic radiation-use efficiency. Response of chlorophyll and carotenoid pigments to stress has been well documented [5-8] and thus is the underlying principle behind remote sensing analysis and

ecological interpretation. The Physiological Reflectance Index (PRI) is based on carotenoid pigments conversions [9] under excess light conditions and has been successfully used to indicate physiological changes from acute [10-11] and chronic stress [5, 13] .

Environmental stressors are a major constraint to the productivity and distribution of plants through limiting photosynthesis. In coastal systems, salinity is considered to be the primary environmental factor influencing community patterns and creating distinct zones of vegetation across the landscape [14-16]. Drought, high irradiance and high temperatures are among many other factors that limit plant growth in these environments [15]. These physical forces create distinct zones of vegetation across the coastal landscape relative to distance from the ocean. These steep environmental gradients cause variations in salinity stress which has been demonstrated as changes in PRI across the landscape [17-18].

Our objective was to evaluate spatial variations in PRI and chlorophyll responses in woody shrubs across a coastal landscape and link to laboratory salinity and drought experiments to understand the physiological mechanisms which allow for survival in a highly dynamic environment. Prior studies indicated that some species do not breakdown chlorophyll in response to stress. Specific goals were to (1) quantify physiological responses and functionality of the photosynthetic apparatus to salinity and drought induced stress in laboratory studies and (2) to use remote sensing methods to understand mechanisms for energy dissipation in the absence of chlorophyll breakdown and relate back to studies in the field.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL AND STUDY SITE

The field studies were conducted summer of 2008 on Hog Island (37° 40'N; 75° 40'W), a barrier island located on the Eastern Shore of Virginia. The oceanside, northern end of the island has been accreting approximately 5 m/year for 140 years [19], resulting in a parallel series of dunes and swales. All field data were collected on the northern end of the island, focusing on three shrubs. *Myrica cerifera* L. (Myricaceae; recently renamed as *Morella cerifera*) is the dominant woody species on many Atlantic coast barrier islands and forms dense, monospecific thickets. *Myrica* thickets leaf area index (LAI) values often exceed most temperate woody communities; above values at which most reflectance indices become insensitive to the background effect of the soil, thus is a model species for scaling up in natural to landscape levels in coastal ecosystems. *Baccharis halimifolia* L. (Asteraceae) is a deciduous generalist small tree or shrub found in swale environments with groundwater salinity ranging from 2 – 5 g L^{-1} and variable soil chlorides [20]. *Iva frutescens* L. (Asteraceae) is a salt-succulent shrub most common along the edge of salt marshes [15, 20]. Seeds of plants were collected on site and germinated in growth chambers for laboratory experiments.

2.2. FIELD MEASUREMENTS

Two sites for each species were selected based on exposure to salinity, one located nearest the Atlantic Ocean with high salt spray exposure, and one interior to the island representing a more protected site. Ten plants per site were selected for measurements.

Leaf samples were collected by punching forty 0.32 cm^2 disks from each plant. Chlorophyll concentrations were determined based on Šesták [21] by extracting chlorophyll using a 100% acetone solution. Samples were ground with a mortar and pestle, filtered, and analyzed using a Spectronic 21 spectrophotometer. Chlorophyll concentrations were calculated using equations given by Holm [22].

Leaf samples from each plant were collected and analyzed for tissue chlorides. Plants were oven-dried at 80°C for 72 h and then ground with a mortar and pestle. For each sample, 0.5 g of material was placed in a tube with 40-mL of deionized water. Samples were placed in a boiling water bath for 2 h, cooled, and filtered into 100-mL volumetric flasks. To each sample, 2-mL of 5 M $NaNO_3$ was added as an ionic equalizer, and then samples were brought to volume with deionized water [20]. Chloride levels were determined using a chloride electrode (model 9617b, Orion, Boston, MA).

2.3. AIRBORNE ACQUISITION

The airborne hyperspectral mission was flown concurrent with the field measurements at Hog Island on August 19, 2008. Air temperature was 30 ºC, with relative humidity of 79% and 2031 μmol m^{-2} s^{-1} PPFD at solar noon. Hyperspectral data (3 nm resolution) were provided by the SpectIR using the ProSpecTIR VIS hyperspectral imaging spectrometer (SpectIR Corp.). Hyperspectral imagery covering 400-1000 nm was collected under cloud-free conditions at 1700 m (AGL) providing a data set representing 1 m / pixel on the ground and a final spectral cube 128 bands deep. These data products were post-processed to correct for geometric and radiometric (e.g., bi-directional) effects. Ground reflectance radiometry was used to calibrate the data based on target endmembers collected in-scene with the ASD FieldSpec Pro Full Range reflectance radiometer (Analytical Spectral Devices, Inc., Boulder, CO). PRI was calculated from pixels as:

$$PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}} \qquad (1)$$

2.4. LABORATORY STUDIES

Salinity treatments of 0 and 15 g L^{-1} were prepared using dilutions of a commercial mixture that approximates total ocean salts (Instant Ocean, Aquarium Systems, Mentor, OH; [23]). Plants were watered daily with the respective saline solution. Measurements were conducted every 3-5 days on plants for 23 days as this timeframe was expected to cause reductions in physiology without mortality [23]. Drought stress was induced by withholding water from plants and comparing to well-watered control plants.

Rates of stomatal conductance (g_s) and leaf net photosynthesis (A_{Net}) were measured using a LI- 6400 (LI-COR Biosciences, Inc., Lincoln, NE) portable infrared gas analyzer on fully expanded leaves from the upper canopy at mid-day (1000 and 1200 solar time). Mid-day xylem pressure potentials (ψ) were quantified with a Scholander pressure chamber (PMS 650, Corvallis, OR).

Chlorophyll fluorescence parameters were measured on upper canopy fully expanded leaves with a pulse amplitude modulated leaf chamber fluorometer (LI-6400, LI-COR Biosciences, Inc., Lincoln, NE). Light-adapted measurements were conducted concurrent with gas exchange measurements.

An ASD FieldSpec Pro reflectance radiometer (Analytical Spectral Devices, Inc., Boulder, CO) was used to measure the spectral reflectance of plant canopies between 350 nm – 2500 nm. The ASD spectral resolution is approximately 1 to 3 nm from the visible to the short-wave infrared. Canopy measurements were collected using a 3200° K lamp as an illumination source. The fore-optic of the radiometer was fixed nadir at a distance of 0.25 meter using an 8° field-of-view. Reflectance spectra were calculated by dividing the spectral radiance by a NIST Spectralon reflectance standard.

At the end of each experiment, pigment and chloride concentrations were determined using the aforementioned methods.

3. RESULTS

3.1. FIELD MEASUREMENTS

Total chlorides present in leaves were highest for the salt succulent plant, *I. frutescens* compared to *M. cerifera* or *B. halimifolia* (Fig. 1). For all species, tissue chlorides were significantly lower at protected sites compared to more exposed sites (Fig 1). Regardless of changes in chlorides, there was no spatial variation in total chlorophyll content (Fig. 1), chlorophyll *a:b*, total carotenoids, or other pigments (data not shown). PRI was significantly different for each species at the respective sites. PRI was lower at more stressed sites from both airborne calculated values (Fig 1) and canopy level values (data not shown). Chlorophyll indices calculated from airborne data did not show any significant differences between sites (data not shown).

3.2. LAB EXPERIMENTS

Stomatal conductance and photosynthesis decreased in response to both salinity and drought. At the end of the drought cycle (Table 1), stomata were completely closed, while some level of opening was maintained due

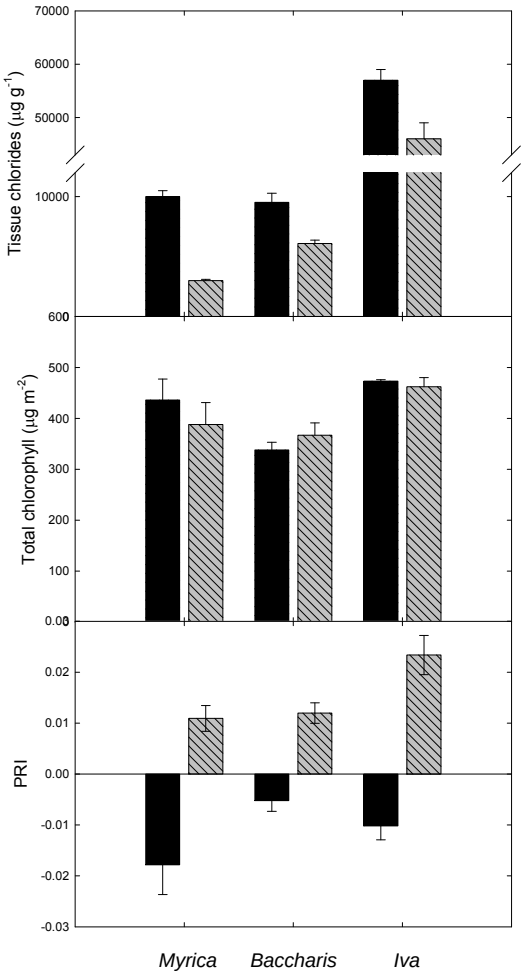

Figure 1. Tissue chlorides (top), chlorophyll content (middle) and PRI (bottom) for three species at an exposed stressed site (solid black bars) and a protected less stressed site (cross-hatched gray bars). Values are expressed as means ± 1 standard error.

Table 1. Physiological measurements, chlorophyll fluorescence and reflectance parameters, and pigment contents for control and stressed *Myrica cerifera* plants at the end of experiments.

Myrica cerifera				
	Drought Stress		Salinity Stress	
	Control	Drought	0 g L^{-1}	15 g L^{-1}
g_s (mmol H_2o $m^{-2}s^{-1}$)	211 ± 53	0.0 ± 0.0*	141 ± 15	0.66 ± 1.5*
A_{net} (µmol CO_2 $m^{-2}s^{-1}$)	10.92 ± 0.99	0.0 ± 0.0*	10.25 ± 0.29	0.66 ± 0.19*
Ψ (MPa)	-0.86 ± 0.04	-1.64 ± 0.22*	-0.76 ± 0.06	-1.82 ± 0.07*
$\Delta F/ F'_m$	0.76 ± 0.00	0.73 ± 0.01*	0.76 ± 0.00	0.62 ± 0.04*
F_v/F_m	0.82 ± 0.00	0.82 ± 0.01	0.82 ± 0.00	0.80 ± 0.00
qP	0.56 ± 0.01	0.17 ± 0.03*	0.57 ± 0.02	0.22 ± 0.002*
qN	0.22 ± 0.07	0.32 ± 0.00*	0.21 ± 0.00	0.28 ± 0.00*
ETR	69.50 ± 1.61	17.06 ± 3.24*	70.16 ± 2.52	18.29 ± 0.98*
PRI	-0.011 ± 0.004	-0.019 ± 0.003*	-0.004 ± 0.003	-0.045 ± 0.003*
Chl *a* (mmol m^{-2})	224 ± 16	218 ± 17	215 ± 29	208 ± 15
Chl *b* (mmol m^{-2})	185 ± 11	171 ± 16	175 ± 21	205 ± 11
Total Chl (mmol m^{-2})	409 ± 26	389 ± 33	390 ± 50	413 ± 24
Chl *a*:*b*	1.2 ± 0.03	1.3 ± 0.05	1.21 ± 0.03	1.01 ± 0.04*
Carotenoids (mmol m^{-2})	33 ± 3	34 ± 4	29 ± 4	25 ± 2
Car:Chl	0.08 ± 0.00	0.09 ± 0.01	0.09 ± 0.01	0.06 ± 0.01
Tissue Chlorides (µg g^{-1})	-	-	4772 ± 320	21887 ± 1427*

to salinity (Table 1). Photosynthetic yield ($\Delta F/F'_m$) decreased more dramatically with salinity stress (from 0.76 to 0.62; Table 1), yet this decrease is not as pronounced as decreases seen in other species due to stress. Drought stress did not lower $\Delta F/F'_m$ as much.

Electron transport rate (ETR) was impaired due to both salinity and drought stress (Table 1). Photochemical quenching (qP) was significantly lowered by both stressors while non-photochemical quenching (qN) did not increase dramatically as typically seen in other studies. There was a significant increase in qN due to both stressors but this was related to PRI only for salinity ($r^2 = 0.727$), not drought ($r^2 = 0.002$). Similar results were found with *B. halimifolia*.

We found no differences in chlorophyll, carotenoid pigments or ratios due to either repeated drought cycles or over a range of salinity concentrations (Table 1).

4. DISCUSSION

Results of our field and laboratory studies show that chlorophyll content did not change in response to stress, despite physiological changes. *Myrica cerifera* had a noticeable different physiological response from other species in response to stress in that it exhibited a slow and steady decline in stomatal conductance, and in doing so it maintained a narrow range of water potentials which enables survival due to episodic stress in a highly variable environment. Stomatal control over photosynthesis was evident in all species.

$\Delta F/F'_m$ declined with increased stress; however, the lowest values were still relatively high compared to values reported in other species [8, 24-25]. While net photosynthetic rate declined, stomata were still open under saline conditions, allowing for some gas exchange. Steady-state fluorescence measurements (data not shown) indicate that despite reduced CO_2 diffusion and prolonged declines in net photosynthesis, PSII remains largely undamaged in both species, with additional mechanisms providing photoprotection. Values of qN at the end of experiments were increased due to stress, but again this change was relatively low compared to other values documented due to stress [7]. This indicates that non-photochemical quenching is one photoprotective mechanism; however this alone may not fully explain the stable chlorophyll contents seen. Increased photorespiration may be a potential mechanism for avoiding photodamage in these plants, since some degree of stomatal conductance was maintained throughout experiments [26].

There was no relationship between PRI and various pigment contents in both laboratory and field studies. PRI has been correlated to plant water status under drought conditions [27-28]. In past studies, spatial variations in PRI and $\Delta F/F'_m$ were not linked to variations in water content during, but did relate to tissue chlorides across the island [17]. In this study, PRI was related to tissue chlorides for all species, while there were no noticeable changes in water content (data not shown). Laboratory experiments demonstrated that PRI was related to qN due to salinity stress, but not drought. This confirms that changes in PRI seen in the field are more likely the result of salinity stress across the landscape. Further, PRI was not related to changes in pigments as there were no significant differences in pigments due to location on the landscape.

Frequently, changes in chlorophyll and carotenoid content are reported in response to various environmental stresses [7, 29-30]. Although this is common, there are studies in which declines are not seen even while variations in PRI occur, especially due to drought [27]. We found no differences in chlorophyll or carotenoid pigments both in laboratory or field studies due to increased concentrations of salinity. These results are less common; however, Redondo-Gómez et al [31] did report similar findings in a succulent halophyte in the presence of various salinity treatments.

Our results indicate that some species do not breakdown chlorophyll during periods of stress, which may be an adaptation a highly dynamic environment. Variations in PRI were not related to changes in chlorophyll content or the carotenoids/chlorophyll ratio as has been demonstrated in other systems. Yet, PRI is an indicator of chronic salinity stress and may be used as an early signal for associated changes due to sea-level rise associated with climate change. Remote sensing of vegetation allows for providing biophysical measurements across landscapes with limited access. However, not all species respond via chlorophyll changes with moderate to high levels of stress and this must be considered as remote sensing of plant signals is applied globally across species.

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